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TUMORIGENESIS BY A FERROACTINOLITE MINERAL

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SUMMARY

In lifetime exposure of male Fischer-344 rats to ferroactinolite fibers* and to UICC amosite asbestos fibers by means of intratracheal and intrapleural treatments, oncogenesis was greater in the lung for the ferroactinolite and in the pleura for the amosite. The lack of correlation between the effects of the two methods of exposure suggests that in this instance, intrapleural inoculation was not a good predictor of pulmonary response on the basis of mass dose. Another feature of the ferroactinolite was that pleural tumors resulted from intratracheal instillations. Conversely, lung tumors or tumorlike lesions were also induced by intrapleural inoculations of ferroactinolite. These facts suggest a greater in vivo transport for the ferroactinolite than for the amosite.

Since there are far fewer mineral fibers per mass unit in the ferroactinolite the tumor yield per unit of mineral fibers was strikingly greater by both routes of administration for ferroactinolite than for amosite.

INTRODUCTION

Nonasbestos mineral fibers are rather commonly present in metamorphic rocks and thus may be present in dusts from mining, stone crushing and from secondary roads. While the deleterious effects of commercial asbestos are well known, the possible hazard of nonasbestos rock fragments is at this time merely speculative, based on similarities to asbestos in the size and shape of the individual particles. A thorough understanding of the biological effects of these nonasbestos minerals, as related to their mineralogical characteristics, is needed as a basis for determining whether a real hazard exists and, if so, what control measures are needed.

*The term 'fiber' refers to mineral particles having parallel sides and a length to width (aspect) ratio > 3.

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The following report describes the first set of a series of experiments relating biological effect of minerals to their mineralogical characteristics. A more detailed discussion of the mineralogical aspects is included in a separate paper [10].

MATERIALS AND METHODS

Test fibers were prepared from loose surface iron-formation rocks. The resulting fibers were identified as ferroactinolite. These were compared to standard UICC amosite fibers by intratracheal instillation and intrapleural inoculation of rats. Mineralogical characterization was as follows [10].

Ferroactinolite

The estimated mineral particle content by volume was as follows: ferroactinolite fibers (50%), sheet silicate plates (20%), magnetite (5%), ferroactinolite and hornblende fragments (20%), and other minerals (5%). The elemental composition of ferroactinolite fibers was silicon, iron, calcium, magnesium, and manganese in order of decreasing amount. The ferroactinolite fiber crystal structure was monoclinic amphibole. The sizes (μm) of the fibers were as follows:

	Mean	Median	Range
Length	3.18	1.50	0.3 – 52.5
Width	0.41	0.24	0.03– 5.23
Aspect ratio	9.0	6.0	3.0 –130.0

UICC amosite

The estimated mineral particle content by volume was follows: grunerite fibers (92%), grunerite fragments (6%), and quartz and other minerals (2%). The elemental composition of amosite was silicon, iron, magnesium, and manganese in order of decreasing amount. The amosite fiber crystal structure was monoclinic amphibole. The sizes of the fibers (μm) were as follows:

	Mean	Median	Range
Length	3.44	1.00	0.15– 378.0[a]
Width	0.29	0.22	0.02– 4.08
Aspect ratio	11.8	7.5	3.0 –2825.0

[a]Amosite fibers up to 600 μm long were observed in areas of electron microscopic preparations not counted.

Specific pathogen-free male Fischer-344 rats were exposed to the test minerals by intratracheal instillations and intrapleural inoculation.

Intratracheal instillation. Twelve weekly injections of 0.5 and 0.25 mg each of ferroactinolite and amosite, respectively, were instilled. The material was suspended in physiological saline containing 0.25% gelatin. The actual dose by mass unit and number of fibers is given in Table I. For study of early pathological sequences and for evaluation of clearance and fate of mineral fibers by electron microscopy, the animals were killed at various intervals up to 1 year. For the lifetime studies for comparison of tumor incidence, rats were permitted to die spontaneously or in rare instances killed in extremis. For evidence of tumor induction, the animals were maintained through their lifespan.

Intrapleural injections. A single injection of 20 mg of each mineral was administered via injection through the chest wall. The dose in number of fibers is given in Table I. The animals were maintained through their lifespan for determination of tumor induction. Tissues were preserved in 10% buffered formalin processed by routine histological procedures. Sagittal sections were prepared including both lung and associated mediastinal tissues, mounted on a single slide and stained with hematoxylin and eosin.

TABLE I
DOSE SCHEDULE BY MASS UNITS AND NUMBER OF FIBERS[a]

Mode of treatment	Dose (mg)	Number of doses	mg per total dose	Fibers per total dose	
				Mineral fibers[a] ($\cdot 10^9$)	Fibers $\geq 8\ \mu m \times \leq 0.25\ \mu m$ ($\cdot 10^6$)
Intrapleural					
Ferroactinolite	20	1	20	1.62	1.8
Amosite	20	1	20	11.4	16.6
Intratracheal					
Ferroactinolite	0.50	12	6.0	0.49	0.54
Amosite	0.25	12	3.0	1.71	25.0

[a]The term 'fiber' refers to mineral particles having parallel sides and length-width (aspect) ratio >3.

RESULTS

Intratracheal instillation

Early sequence studies. Observation by histopathology showed granulomatous changes in the bronchi for both fibers at the first killing period (after the 12th instillation). These consisted of large polypoid granulomatous lesions in the smaller airways with numerous fiber-containing solitary macrophage and giant cells. These masses were invested with normal-appearing ciliated epithelium. This lesion was

characteristic of the amosite-treated animals but occurred infrequently and were much smaller in the ferroactinolite treatment. These lesions regressed with time and few were evident at 1 year. A second lesion consisting of sporadic foci of interalveolar septal thickening adjacent to the terminal bronchiolar-alveolar ductal junctures was barely evident at the first killing period following the last of the 12 weekly intratracheal instillations but became more pronounced at the later killing periods. There was gradual alteration to higher epithelium, one animal showing frank bronchiolo-alveolar-cell hyperplasia by the termination of the experiment (1 year). Squamous metaplasia was also evident in a few of these lesions. This lesion was more common and the epithelial changes more evident for the ferroactinolite treatment, persisting through the lifetime studies, below.

Lifetime intratracheal studies. Bronchiolo-alveolar-cell tumors and atypical bronchiolo-alveolar-cell hyperplasia were the most common lesions.* These lesions were frequently multiple, and appeared to intergrade sometimes in the same lung. Squamous metaplasia also developed in the hyperplastic lesions. A few were frankly carcinogenic. Other tumors noted consisted of adeno and epidermoid carcinoma. The incidence of lung tumors in ferroactinolite, amosite, sham, and untreated controls were 3.9, 0.71, 0.6, and 0.4%, respectively. The number of hyperplastic lesions were similar in the two treatments 6.6 and 7.9%, respectively. There was also a low incidence of mesothelioma in the two treatment groups. In addition, there were 2.1% metastatic tumors in the ferroactinolite group as compared to 0, 0.94, and 0.46% in the amosite, sham, and untreated groups. Primary sites when identifiable were testis, salivary gland, urinary bladder, and neck (Table II).

Statistical evaluation by Fisher exact T test revealed that the incidence of lung tumors was significant in ferroactinolite group compared to amosite and the controls ($P = 0.05$). The incidence of atypical cell hyperplasia was not significant in the two groups when compared to each other. The incidence, however, was significant when compared with the controls ($P < 0.005$). The incidence of mesotheliomas and metastatic tumors was not significant.

Intrapleural inoculation

Mesotheliomas were noted in both amosite and ferroactinolite treated animals with a slightly higher incidence for amosite 33.6 and 23%, respectively. The mesotheliomas consisted of fibrosarcomatous, tubulopapillary, pleomorphic and mixed types. The fibrosarcomatous mesotheliomas were most common, aggressively invading the lungs, mediastinal structures, and the chest walls. The second lesion observed in the treated animals was granuloma; 20 and 30.7% in ferroactinolite and amosite treated animals respectively (Table III).

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*The classification of the lung and pleural lesions observed in this study are based on the consensus of an expert review panel of six pathologists convened by Universities Associated for Research and Education in Pathology (UNREP).

TABLE II
SUMMARY OF PLEURAL AND LUNG LESIONS
Intratracheal instillation.

Treatment	Ferroactinolite	Amosite	Sham	No treatment
Number of animals	561	139	212	219
Neoplastic lesions				
Pleura				
Sarcomatous mesothelioma	1	1	0	0
Tubulopapillary mesothelioma	2	1	0	0
Mixed mesothelioma	2	0	0	0
Pleomorphic mesothelioma	0	0	0	0
Total	5	2	0	0
Lung (primary)				
Epidermoid carcinoma	7	1	0	0
Bronchioloalveolar tumor	8	0	0	0
Adenocarcinoma	5	0	2	1
Adenoma	2	0	0	0
Total	22	1	2	1
Lung (secondary)				
Metastatic tumors	12	0	2	1
Other lesions				
Lung				
Atypical bronchioloalveolar cell hyperplasia	37	11	0	3
Granuloma	0	0	0	0

Statistical analysis of the data by Fisher exact T test revealed that the incidence of mesothelioma and granuloma in comparison within the two treated groups was not statistically significant. When the incidence was compared with the controls, however, it was highly significant ($P<0.0001$)

Fate of mineral fibers

Examination by transmission electron microscopy of low temperature ashed whole lung specimens of animals killed sequentially indicated that the mineralogical characteristics of both ferroactinolite and amosite fibers changed with time. Longitudinal splitting of the fibers resulted in a greater number of thinner fibers with increased aspect ratio. This resulted in an absolute increase of number for ferroactinolite but a relative decline in the number of amosite fibers probably because removal by clearance exceeded the increase by splitting [10].

TABLE III
SUMMARY OF PLEURAL AND LUNG LESIONS
Intrapleural inoculation.

Treatment	Ferroactinolite	Amosite	Sham	No treatment
Number of animals	135	137	134	219
Neoplastic lesions				
Pleura				
Sarcomatous mesothelioma	21	31	0	0
Tubulopapillary mesothelioma	3	2	2	0
Mixed mesothelioma	5	12	0	0
Pleomorphic mesothelioma	2	1	0	0
Total	31	46	2	0
Lung (primary)				
Epidermoid carcinoma	0	0	0	0
Bronchioloalveolar tumor	1	1	0	0
Adenocarcinoma	1	0	0	1
Adenoma	0	0	1	0
Total	2	1	1	1
Lung (secondary)				
Metastatic tumors	0	0	0	1
Other lesions				
Lung				
Atypical bronchioloalveolar cell hyperplasia	4	0	0	3
Granuloma	27	42	0	0

DISCUSSION

The data demonstrate that ferroactinolite produced neoplastic lesions through both routes of inoculation. On the basis of mass dose by intratracheal instillation oncogenic potency was greater for the ferroactinolite, whereas, by intrapleural inoculation potency was greater for the amosite, however, the difference was not statistically significant. This suggests that under the conditions of this experiment the intrapleural route was not a good predictor of pulmonary tumors.

The apparent difference in the induction of neoplastic lesions in the respective organs by the two minerals is of interest because data are often considered from pleural implantation [1] or intrapleural inoculation [2, 3] for the assessment of potential carcinogenicity of the mineral. While such data are of basic importance for mechanistic purposes they are artificial and do not represent direct exposure of the actual minerals to lung.* Our data suggest that more attention should be given

*Other material such as plastic discs are known to produce tumors when directly introduced into the pleura. This phenomenon is sometimes termed solid-state carcinogenesis [9]. It will also be noted that two mesothelial tumors occurred in our sham-treated controls possibly attributable to the gelatin used as a vehicle.

to inhalation and intratracheal instillation studies with intrapleural methods reserved for first-order tests since other factors such as the ability of the fibers to penetrate tissue may not play a pathogenic role by this route of administration. It is, however, recognized that previous investigators who had employed intratracheal instillation for tumorigenesis evaluation of asbestos, with one exception [4], met with limited success. A significantly high incidence of lung tumor was, however, observed when the fibers were inoculated in conjuction with polycyclic hydrocarbons [5–7].

Stanton et al. [8] have demonstrated that induction of mesothelial tumors from intrapleural introduction of inorganic particles appears dependent on the presence of long thin fibers with the optimal effect taking place with fibers of ≥ 8 μm long and ≤ 0.25 μm wide. Our data for pleural tumors appear inconsistent when compared to the excess of mineral fibers for amosite (92 times that of ferroactinolite for fibers ≥ 8 μm $\times \leq 0.25$ μm). Our data, however, indicate only 4.5 times more tumors in the amosite group. If fiber dimension were equally determinative for the lung, the differences would have been even larger, for here the carcinogenicity of the ferroactinolite was actually much greater than that of amosite.

The most probable explanation of these incogruities is in vivo splitting of fibers in the lung. This phenomenon increased the number of fibers and aspect ratio thus increasing the dose during the residence of the lung [10].

REFERENCES

1 M.F. Stanton, Some etiological considerations of fibre carcinogenesis, in Biological Effect of Asbestos, Proc. IARC Lyon, 1972, pp. 289–294, IARC Sci. Publ. No. 8.

2 J.C. Wagner, Induction of experimental tumors of the pleura by fibers (asbestos) Oncol. Proc. 10th Int. Cancer Cong., 1970, pp. 446–451.

3 W.E. Smith, L. Miller, R.E. Elsasser and D.D. Hubert, Test for carcinogenicity of asbestos, Ann. N.Y. Acad. Sci. 132 (1965) 456–488.

4 A. Küng-Vösamae and F. Vinkman, Combined carcinogenic action of chrysotile asbestos dust and *N*- Nitrosodiethylamine on the respiratory tract of Syrian golden hamster, in J.C. Wagner (Ed.), Effects of Mineral Fibers, 1980, pp. 305–310.

5 W.E. Smith, L. Miller, J. Chury and I.J. Selikoff, Pleural reaction and mesothelioma in hamsters injected with asbestos, Proc. Am. Assoc. Can. Res., 5 (1964) 59.

6 L.M. Shabad, Experimental cancer of the lung. J. Natl. Cancer Inst., 28 (1962) 1305–1332.

7 L.N. Pylev and L.M. Shabad, Some results of experimental studies in asbestos carcinogenesis, in Biological Effects of Asbestos, Proc. IARC, Lyon, 1972, pp. 99–105.

8 M.F. Stanton, M. Layard, A. Tegeris, E. Miller, M. May and E. Kent, Carcinogenicity of fibrous glass: pleural response in the rat in relation to fiber dimension, J. Natl. Cancer Inst., 58 (1977) 587–603.

9 J. Autian, A.R. Singh, J.E. Turner, G.W.C. Hung, L.J. Nunez, and W.H. Lawrence, Carcinogenesis from polyurethans, Cancer Res., 35 (1975) 1591–1596.

10 P.M. Cook, L.D. Palekar and D.L. Coffin, Interpretation of the carcinogenicity of amosite asbestos and ferroactinolite on the basis of retained fiber dose and characteristics in vivo, Toxicol. Lett., 18 (1982) 151–158.